OsteoArthritis and Cartilage (2007) **15,** 245–250

doi:10.1016/j.joca.2006.08.009

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

The validity of *in vitro* ultrasonographic grading of osteoarthritic femoral condylar cartilage – a comparison with histologic grading[1]

C.-Y. Tsai M.D.†, C.-L. Lee M.D., M.S.†, C.-Y. Chai M.D., Ph.D.‡§, C.-H. Chen M.D.†‖, J.-Y. Su M.D.¶#, H.-T. Huang M.D.¶# and M.-H. Huang M.D., Ph.D.†‖*

† *Department of Physical Medicine and Rehabilitation, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*

‡ *Department of Pathology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*

§ *Department of Pathology, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*

‖ *Department of Physical Medicine and Rehabilitation, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*

¶ *Department of Orthopedic Surgery, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*

Department of Orthopedic Surgery, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

Summary

Objective: To establish an ultrasonographic grading for semi-quantitative evaluation of the femoral condylar cartilage of knee osteoarthritis (OA), *in vitro*, and compare the ultrasonographic grading with the histologic grading.

Design: Thirty-four patients going to receive total knee arthroplasty because of OA of the knee were recruited. Specimens of the distal medial and lateral femoral condyles were taken during the operation. The anterior and middle areas of the articular cartilage in each specimen were graded by *in vitro* ultrasonography (US) examination and histologic evaluation. The correlation between the US and histologic findings was analyzed.

Results: Sixty-seven specimens were collected. Both US and histologic changes were graded from I to IV. The correlation between the grading of US and histology was good in both anterior and middle areas (Rho = 0.78, 0.89, $P < 0.001$). The correlation between US grading over anterior area and histology grading over middle area was moderate (Rho = 0.70, $P < 0.001$).

Conclusions: The moderate correlation between *in vitro* US and histology might permit quantitative *in vivo* assessment of cartilage.

Key words: Osteoarthritis, Articular cartilage, Ultrasonography, Histology.

Introduction

Osteoarthritis (OA) is a severe musculoskeletal disease, commonly diagnosed in elderly people[1]. OA of the knee is particularly prevalent and people suffer due to pain, stiffness, and muscle weakness[2,3]. If conservative symptomatic treatment cannot relieve the pain, patients have to receive surgical intervention such as total joint arthroplasty. However, surgical implants are not a satisfactory procedure because implants have a limited life span and limited tolerance for heavy loading and vigorous activity[4]. OA is a progressive degradation of the articular cartilage. Relative to the collagen in the matrix, the earliest change in the OA cartilage is diminution of mucopolysaccharide chondroitin sulphate. This results in depletion of the ground substance and unmasking of the collagen. The injury of the collagen network is thought to represent the "point of no return" in the process of OA progression[5]. There are several methods suggested for treating early knee OA, such as intra-articular injection of hyaluronic acid and taking glucosamine[6–8], etc. Therefore, early detection of the pathologic changes in cartilage is essential in order to treat the disease successfully[9].

Current modalities for knee OA diagnosis include non-invasive techniques, such as plain film, computed tomography (CT), magnetic resonance imaging (MRI) and ultrasonography (US), and invasive techniques, such as arthrography and arthroscopy. Plain film demonstrates osseous abnormalities but is insensitive for detecting lesions of soft tissue such as articular cartilage[10]. The CT images can be obtained in the transverse plane, which is perpendicular to the direction of weight bearing and in which cartilage over the weight bearing area cannot be visualized. Although the image reconstruction in the sagittal and coronal planes is available, the resultant image quality is inferior to those obtained by a direct imaging technique[11]. Arthrography and arthroscopy are not ideal screening tests

[1]This study was granted by Kaohsiung Medical University, M094019.

*Address correspondence and reprint requests to: Mao-Hsiung Huang, M.D., Ph.D., Department of Physical Medicine and Rehabilitation, Kaohsiung Medical University Hospital, No. 100 Tzyou 1st Road, Kaohsiung 807, Taiwan. Tel: 886-7-3208211; Fax: 886-7-3208211; E-mail: maohuang@ms24.hinet.net

Received 5 May 2006; revision accepted 19 August 2006.

because they need either a fluoroscopy suite or an operation room and both of them are invasive procedures[12]. Besides, arthroscopy reveals only macroscopic changes over the surface of degenerated cartilage. MRI is a powerful tool for thorough evaluation of the articular cartilage. However, there are several technical and economic limitations for routine use in assessing osteoarthritic cartilage[11,13]. Nuclear medicine study has also been used for early detection of OA, but the patient is exposed to radiation during the examination[14].

US is a quick, inexpensive and non-invasive method for articular cartilage assessment[15]. It plays an important role in imaging the musculoskeletal system to detect the minimal change of soft tissue[16,17]. Several studies had used US to assess the thickness and surface characteristics of articular cartilage[9,18–20]. Although US is a skill-dependent method, Disler *et al.*[21] showed that US was accurate and reliable for detection and grading of knee articular cartilage defects. W. Joseph McCune only graded the clarity and sharpness of the femoral condylar cartilage individually and made a correlation with gross operative findings[22]. It still lacked a US grading of OA cartilage using all the US characteristics including homogenicity of cartilage, sharpness of superficial and deep margin and narrowing of cartilage band. Further its correlation with histologic change is also lacking. The aim of this study was to establish a semi-quantitative method to evaluate the femoral condylar cartilage by *in vitro* US grading, and compare with its histologic grading.

Material and method

SUBJECTS

Thirty-four patients who had a diagnosis of knee OA and were going to receive total knee arthroplasty were recruited. All of them were classified equal to or greater than Ahlbäck stage III[23] and most of the lateral compartments of the femoral condyle were less severe than medial compartments.

SPECIMEN

During the knee arthroplasty operations, distal femoral condyles were excised with an oscillating saw. The distal medial and lateral condyle specimens were chosen and rinsed in saline.

The degeneration of the cartilage is usually inhomogeneous. It is difficult to select an area to represent the whole changes. We selected the areas for examination just according to the location. Two transverse areas on each specimen, including anterior and middle areas, were chosen (Fig. 1). The middle area of the specimen was located over the middle part of the specimen and represented the weight bearing portion of the femoral condyle. The anterior area of the specimen was located over the middle of the anterior part of the specimen and represented the sub-weight bearing portion. Both these areas were marked with gentian violet and evaluated by US and histologic examinations.

US EXAMINATION

Previous studies had described the ultrasonographic characteristics of articular cartilage. The ultrasonographic image of normal articular cartilage is a homogeneous anechoic band. The synovial space–cartilage interface (anterior margin) and the cartilage–bone interface (posterior margin) are sharp[13,24]. In osteoarthritic cartilage, the anterior and posterior margins become blurred or obliterated and the clarity of the cartilage layer is decreased. In patients with more advanced OA, narrowing of the articular cartilage can be found. Nearly complete loss of articular cartilage can be observed in patients with most advanced OA.

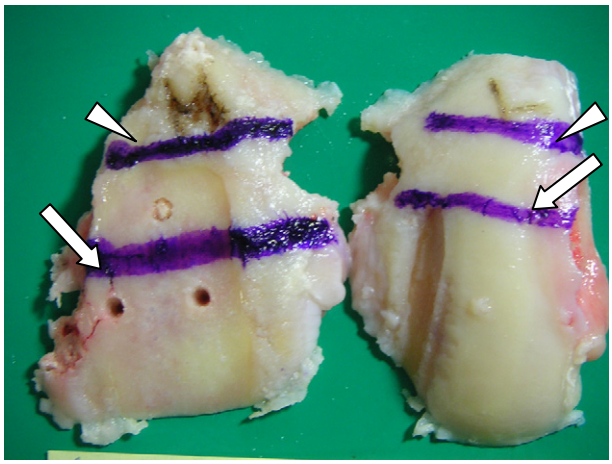

Fig. 1. Two transverse areas, anterior (arrowheads) and middle (white arrows), were chosen on both medial and lateral condyle specimens for US examination. Then they were marked with gentian violet for localization of histologic examination.

We classified the US findings into grades 1–4 according to the above characteristics of OA as Fig. 2. Grade 1 showed a homogenously anechoic cartilage band with sharp superficial and deep margins (we use these terms because the specimens were examined *in vitro*). Grade 2 showed blurring or obliteration of the margin of the cartilage band. Grade 3 included blurring, obliteration of the margin and narrowing of the cartilage band. Grade 4 was coded if the cartilage band could not be visualized.

The specimens were evaluated using a real time 5–12 MHz high-resolution linear transducer (HDI 1500; Advanced Technologies Laboratories, Bethell, WA). During the examination, a US pad was placed between the transducer and the specimen. The transducer was perpendicular to the surface of the specimens. The imaging parameters were set properly without change for the evaluation of all the specimens. The US grading of specimens was made by the same experienced investigator who was blinded to the histologic grading.

HISTOLOGIC EXAMINATION

After US examination, tissue specimens were rinsed and fixed with 4% paraformaldehyde for 24 h and decalcified in a decalcifier for a few days. Then the specimens were processed for embedding in paraffin wax. Standardized 4 μm sections of the specimen were prepared from anterior and middle areas. The sections were stained with haematoxylin and eosin (HE). The characteristics of histologic changes in osteoarthritic cartilage under light microscope are described in Table I[4,25]. Two pathologists, who were blinded to US results, evaluated the histologic changes in the articular cartilage under light microscope. They coded a histologic grading according to these histologic characteristics[4] (Fig. 3).

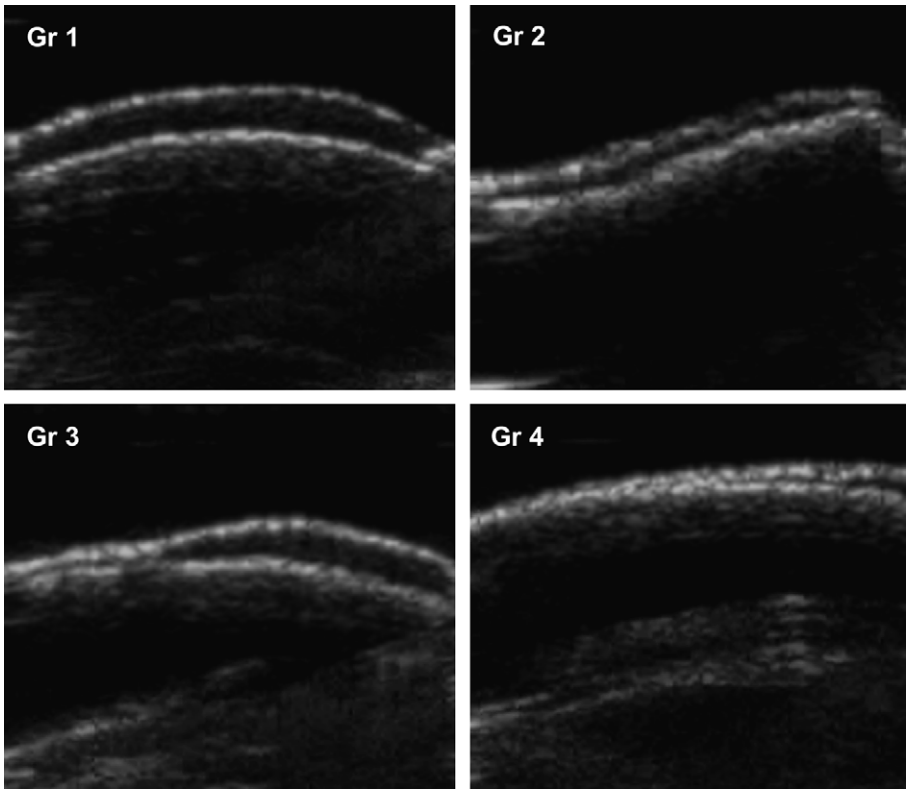

Fig. 2. US grading of OA cartilage. Grade 1: homogeneously anechoic band with sharp margin. Grade 2: blurring or obliteration of the margin. Grade 3: blurring, obliteration of margin and narrowing of cartilage band. Grade 4: cartilage band cannot be visualized.

STATISTICAL ANALYSIS

Spearman's correlation was performed to determine the correlation between *in vitro* US grading and histologic grading of articular cartilage. A significant correlation was defined as P value less than 0.05. A Rho score of less than 0.40 indicated poor agreement; that of 0.40−0.75, moderate agreement; and that of 0.75−1.00, good agreement.

Results

Thirty-four patients were recruited in this study. Each arthritic knee offered two specimens. One specimen was excluded due to poor decalcification. Sixty-seven specimens of femoral condylar cartilage were used for analysis.

THE DISTRIBUTION OF US AND HISTOLOGIC GRADING

The distribution of US grading and histologic grading over various locations is listed in Table II. The cartilage degeneration was less severe in lateral femoral condyles than in medial parts (Table II). The grades of overall specimens, including medial and lateral, ranged from grades 1 to 4.

The distribution of US and histologic grading over the anterior area is shown in Table III, and the distribution of US and histologic grading over the middle area is shown in Table IV.

THE CORRELATION OF US AND HISTOLOGIC GRADING

The correlation of US and histologic grading over anterior and middle areas was good (Rho = 0.78, 0.89, both $P < 0.001$). The correlation of US grading between anterior and middle areas of the specimen was moderate (Rho = 0.70, $P < 0.001$). The correlation of histologic grading between anterior and middle areas was also moderate (Rho = 0.69, $P < 0.001$). In addition, the US grading over anterior area and histologic grading over middle area showed moderate agreement (Rho = 0.70, $P < 0.001$) (Table V).

Discussion

The US feature of normal articular cartilage is a homogeneous anechoic band with a sharp superficial cartilage profile (superficial margin) and subchondral bone profile (deep margin) which is due to the water content of cartilage being high and the cartilage surface being flat and smooth. The earlier US features of OA are loss of clarity of the cartilage

Table I
The characteristic of histologic changes of OA

Characteristic	Finding on light microscope
Flaking	Indentation of cartilage capsule
Fibrillation	Superficial breading of cartilage
Chondrocyte enlargement	Chondrocyte cytoplasm filled with hyaline-like substance in Zones II−IV
Hyalinization	Chondrocyte loss in Zones II−III
Pitting	Irregular surface of cartilage
Cartilage loss	Decreased thickness of cartilage

Zone I: superficial zone; Zone II: mid zone; Zone III: deep zone; Zone IV: calcified cartilage[25].

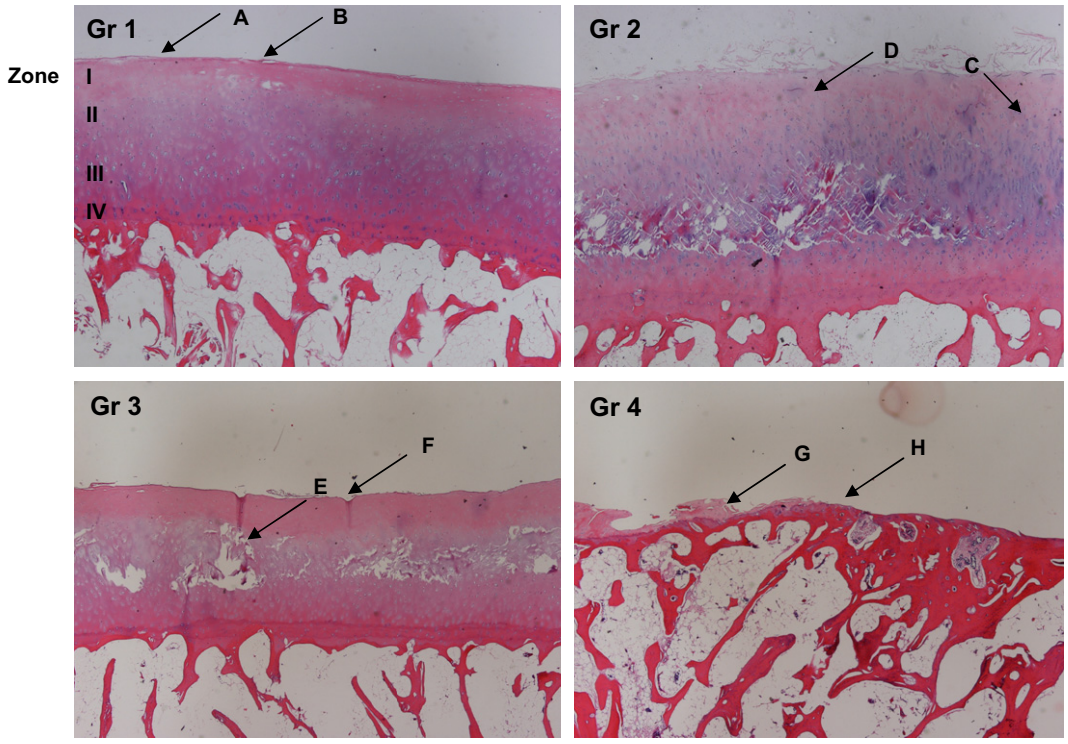

Fig. 3. The histologic grading and characteristics of OA cartilage. Grade 1: flaking (A) and superficial fibrillation (B). Grade 2: chondrocyte enlargement (C) and hyalinization (D). Grade 3: deep fibrillation (E) and pitting (F). Grade 4: partial cartilage loss (G) or complete cartilage loss (H). HE stain, 40×.

band and loss of sharpness of the margin. The loss of sharpness of the interface is due to scattering of sound by a rough surface. The increased echogenicity may represent structural alteration such as fibrillation of cartilage and cleft formation[26]. In the cases with more severe OA, the thickness of cartilage band becomes narrowed and may have disappeared. However, the loss of sharpness of the interface made placement of markers for thickness measurement difficult[22,24].

Disler *et al.*[21] evaluated porcine and human knee specimens. Defects on the porcine knee cartilage were created for surgical grades. The human knee specimens were retrieved from patients who underwent total knee arthroplasty. Both these kinds of specimens were graded using surface observation (surgical grades) and US examination (US grades). Interobserver agreement of US grade was found to be excellent. The concordance of US and surgical grades was also excellent. The study showed that US was reliable for the detection of defects regardless of them being artificial or pathologic. However the US grades used in this study were only determined by the depth of defects in morphological change.

McCune *et al.*[22] used the US features of femoral condylar cartilage, including thickness, clarity and sharpness to evaluate the OA change. They compared the individual US features with operative gross findings. They found the clarity and sharpness of the cartilage images correlated significantly with gross findings of the specimen. They also found that evaluation of US images obtained in the transverse plane showed the best correlation with gross pathologic findings. However, they did not evaluate the specimens by histologic changes which could reveal early changes of OA cartilage.

In the present study, we included all US changes of articular cartilage in the grading system. Further, the histologic changes evaluated by pathologists were used for comparisons which were more related with the deterioration of the arthritic cartilage. We found that the US grades correlated

Table II
Distribution of US and histologic grades over various locations

Grade	US						Histology					
	Anterior area			Middle area			Anterior area			Middle area		
	M	L	Total	M	L	Total	M	L	Total	M	L	Total
1	4	24	28	0	25	25	3	18	21	1	20	21
2	7	6	13	4	5	9	4	10	14	3	7	10
3	13	2	15	15	1	16	19	4	23	11	4	15
4	10	1	11	15	2	17	8	1	9	19	2	21

M: specimen of medial femoral condyle; L: specimen of lateral femoral condyle.

Table III
Distribution of US and histologic grades of distal femoral condyle (anterior area)

US grade	Histologic grade			
	1	2	3	4
1	18	8	2	0
2	1	6	5	1
3	2	0	12	1
4	0	0	4	7

well with histologic grades (Rho = 0.78, 0.89, both $P < 0.001$). The correlation of US findings and histologic changes demonstrated that well controlled ultrasonography might be a valid tool for evaluation of arthritic cartilage changes in OA.

The weight bearing portion of the femoral condylar cartilage was supposed to change first in a case of knee OA. This was the area we were interested in and had to evaluate. The weight bearing portion of femoral condylar cartilage could be well evaluated in patients who could flex their knees adequately[11,18,22]. However, limited range of knee joint motion which made weight bearing portion visualization difficult was quite common in patients with knee OA. Thus, we chose an anterior area of our specimen to represent the anterior sub-weight bearing portion of the femoral condyle where visualization was much easier, *in vivo*. There was moderate correlation between US grading over anterior area and histologic grading over middle area (Rho = 0.70, $P<0.001$). This implied that the US findings from the sub-weight bearing region might be reliable for the evaluation of the arthritic cartilage changes of the weight bearing region in clinical practice. However, several limitations would be met when we evaluated the femoral condylar cartilage, *in vivo*. The US beam would be interfered with by the osseous portion of the patella[12]. Moreover, visualization of the interface of synovial space–cartilage might be impaired in patients with synovitis[13]. Further study on *in vivo* ultrasonographic examination of femoral condylar cartilage is warranted.

In conclusion, this preliminary work revealed good correlation of *in vitro* US grading and histologic grading of femoral condylar cartilage. A moderate correlation was found between US grading over sub-weight bearing areas and histologic grading over weight bearing areas.

Acknowledgments

The authors wish to thank the Statistical Analysis Laboratory, Department of Clinical Research, Kaohsiung Medical University Chung-Ho Memorial Hospital for all their help.

Table IV
Distribution of US and histologic grades of distal femoral condyle (middle area)

US grade	Histologic grade			
	1	2	3	4
1	20	2	3	0
2	1	6	2	0
3	0	2	9	5
4	0	0	1	16

Table V
The correlation between US and histologic grading

	US-A	US-M	His-A	His-M
US-A	–	0.70 $P < 0.001$	0.78 $P < 0.001$	0.70 $P < 0.001$
US-M	0.70 $P < 0.001$	–	0.66 $P < 0.001$	0.89 $P < 0.001$
His-A	0.78 $P < 0.001$	0.66 $P < 0.001$	–	0.69 $P < 0.001$
His-M	0.70 $P < 0.001$	0.89 $P < 0.001$	0.69 $P < 0.001$	–

The data presented in the table is Rho. US-A: US grade over anterior area; US-M: US grade over middle area; His-A: histologic grade over anterior area; His-M: histologic grade over middle area.

The authors also thank Wan-Ting Huang, M.D., Department of Pathology, for evaluating histologic changes of the cartilage, and Ming-Wei Chang, M.D., Department of Orthopedic Surgery, for specimen collection.

References

1. La Valley M, McAlindon TE, Evans S, Chaisson CE, Felson DT. Problems in the development and validation of questionnaire-based screening instruments for ascertaining cases with symptomatic knee osteoarthritis: The Framingham Study. Arthritis Rheum 2001;44:1105–13.
2. McAlindon TE, Cooper C, Kirwan JR, Dieppe PA. Knee pain and disability in the community. Br J Rheumatol 1992;31:189–92.
3. O'Reilly SC, Jones A, Muir KR, Doherty M. Quadriceps weakness in knee osteoarthritis: the effect on pain and disability. Ann Rheum Dis 1998;57:588–94.
4. Huang MH, Ding HJY, Chai CY, Huang YF, Yang RC. Effects of sonication on articular cartilage in experimental osteoarthritis. J Rheumatol 1997;24:1978–84.
5. Buckwalter JA, Mankin HJ. Articular cartilage, Part II. Degeneration and osteoarthritis, repair, regeneration, and transplantation. J Bone Joint Surg Am 1997;79:612–32.
6. Frizziero L, Pasquali Ronchetti I. Intra-articular treatment of osteoarthritis of the knee: an arthroscopic and clinical comparison between sodium hyaluronate (500–730 kDa) and methylprednisolone acetate. J Orthop Trauma 2002;3:89–96.
7. Altman RD, Moskowitz R. Intraarticular Sodium Hyaluronate (Hyalgan®) in the treatment of patients with osteoarthritis of the knee: a randomized clinical trial. J Rheumatol 1998;25:2203–12.
8. Largo R, Alvarez-Soria MA, Díez-Ortego I, Calvo E, Sánchez-Pernaute O, Egido J, *et al*. Glucosamine inhibits IL-1 beta-induced NF kappa B activation in human osteoarthritic chondrocytes. Osteoarthritis Cartilage 2003;11:290–8.
9. Nieminen HJ, Töyräs J, Rieppo J, Nieminen MT, Hirvonen J, Korhonen R, *et al*. Real-time ultrasound of articular cartilage degradation *in vitro*. Ultrasound Med Biol 2002;28:519–25.
10. Tarhan S, Unlu Z, Goktan C. Magnetic resonance imaging and ultrasonographic evaluation of the patients with knee osteoarthritis: a comparative study. Clin Rheumatol 2003;22:181–8.

11. Martel W, Adler RS, Chan K, Niklason L, Helvie MA, Jonsson K. Overview: new methods in imaging osteoarthritis. J Rheumatol Suppl 1991;18:32–7.
12. Richardson ML, Selby B, Montana MA, Mack LA. Ultrasonography of the knee. Radiol Clin North Am 1988; 26:63–75.
13. Grassi W, Lamanna G, Farina A, Cervini C. Sonographic imaging of normal and osteoarthritic cartilage. Semin Arthritis Rheum 1999;28:398–403.
14. Huang MH, Ding HJ, Yang CC, Chai CY, Yang RC. The early evaluation of induced osteoarthritis in rats with technetium-99m pertechnetate scans. Nucl Med Commun 1996;17:529–35.
15. Grassi W, Cervini C. Ultrasonography in rheumatology: an evolving technique. Ann Rheum Dis 1998;57: 268–71.
16. Court-Payen M. Sonography of the knee: intra-articular pathology. J Clin Ultrasound 2004;32:481–90.
17. Harcke HT, Grissom LE, Finkelstein MS. Evaluation of the musculoskeletal system with sonography. Am J Roentgenol 1998;150:1253–61.
18. Jonsson K, Buckwalter K, Helvie M, Niklason L, Martel W. Precision of hyaline cartilage thickness measurements. Acta Radiol 1992;33:234–9.
19. Töyräs J, Nieminen HJ, Laasanen MS, Nieminen MT, Korhonen RK, Rieppo J, *et al*. Ultrasonic characterization of articular cartilage. Biorheology 2002;39:161–9.
20. Adler RS, Dedrick DK, Laing TJ, Chiang EH, Meyer CR, Bland PH, *et al*. Quantitative assessment of cartilage surface roughness in osteoarthritis using high frequency ultrasound. Ultrasound Med Biol 1992;18: 51–8.
21. Disler DG, Raymond E, May DA, Wayne JS, McCauley TR. Articular cartilage defects: *in vitro* evaluation of accuracy and interobserver reliability for detection and grading with US. Radiology 2000;215: 846–51.
22. McCune WJ, Dedrick DK, Aisen AM, MacGuire A. Sonographic evaluation of osteoarthritic femoral condylar cartilage: correlation with operative findings. Clin Orthop 1990;254:230–5.
23. Ahlbäck S. Osteoarthritis of the knee: a radiographic investigation. Acta Radiol 1968;277(Suppl):7–72.
24. Aisen AM, McCune WJ, MacGuire A, Carson PL, Silver TM, Jafri SZ, *et al*. Sonographic evaluation of the cartilage of the knee. Radiology 1984;102:781–4.
25. Pritzker KPH, Gay S, Jimenez SA, Ostergaard K, Pelletier J-P, Revell PA, *et al*. Osteoarthritis cartilage histopathology: grading and staging. Osteoarthritis Cartilage 2006;14:13–29.
26. Myers SL, Dines K, Brandt DA, Brandt KD, Albrecht ME. Experimental assessment by high frequency ultrasound of articular cartilage thickness and osteoarthritic changes. J Rheumatol 1995;22:109–16.